18. (Twice amended) A kit for distinguishing methylated and non-methylated nucleic acid sequences, comprising a labeled oligonucleotide probe, wherein said labeled oligonucleotide probe comprises a fluorophore moiety, a loop sequence, and a quencher moiety, and wherein said loop sequence has a region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation.

REMARKS

Claims 1-18 are currently pending in the application. Claim 18 has been amended. The amendment finds support in the application, drawings and claims as originally filed, and is discussed in the relevant section below. No new matter has been added.

I. Rejection of Claim 18 under 35 U.S.C. § 102(e)

Claim 18 is rejected under U.S.C. § 102(e) as being anticipated by Tyagi, et al., U.S. Patent 6,037,130 (hereinafter "Tyagi '130"). The Examiner states that Tyagi '130 "teaches a kit comprising a detector probe which is a fluorescently labeled hairpin forming oligonucleotides containing a fluorescent emitter and a quencher." The Examiner also notes that Claim 18 comprises open claim language, and therefore is not limited to a probe which contains *only* two moieties (fluorophore and quencher), as suggested in the previous response.

Claim 18 requires a loop sequence which has a "region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation." Although the Tyagi '130 reference teaches a detector probe having both a fluorescent emitter (fluorophore) and a quencher, it does not teach an oligonucleotide probe having "a loop sequence comprising a nucleotide region complimentary to a region of the nucleic acid sample, wherein the region of the nucleic acid sample is susceptible to methylation," as required by Claim 18. Thus, because the Tyagi `130 reference does not teach every element of Claim 18, it does not anticipate the claimed invention.

Thus, the Applicant respectfully requests that the rejection under 35 U.S.C. § 102(e) be reconsidered and withdrawn.

II. Rejections under 35 U.S.C. § 103(a)

A) Rejection of Claims 1-6 and 14-17 under 35 U.S.C. § 103(a)

Reasons for Rejection

Claims 1-6 and 14-17 are rejected under 35 U.S.C. § 103(a) over Elsas, et al., U.S. Patent 6,207,387 (hereinafter "Elsas") in view of either Ehrlich, et al., *Biochimica et Biophysica Acta*, Vol. 395, pages 109-119, 1975 (hereinafter "Ehrlich") or Hua, et al., *Gov. Rep. Announce. Index US*, Vol. 88, No. 18, Abstract No. 847,050 1988 (hereinafter "Hua") and in further view of either Tyagi, et al., U.S. Patent 6,150,097 (hereinafter "Tyagi '097") or Coull, et al., U.S. Patent 6,355,421 (hereinafter "Coull").

The Examiner states that it would have been obvious to modify the method of Elsas for detecting different nucleic acids, with (1) the teachings of either Tyagi '097 or Coull which discuss stem-loop and fluorescence energy transfer, and (2) the teachings of Ehrlich or Hua which discuss the properties of methylated DNA. Applicant respectfully disagrees with the grounds of the rejection.

Response to the Rejection

The teachings of the cited references are discussed in detail in the Amendment and Reply dated October 23, 2002, which is hereby incorporated by reference in its entirety. Briefly, Elsas teaches a process for detecting "missense" or "nonsense" mutations in the gene responsible for galactosemia. Ehrlich investigated the physical and chemical properties of bacteriophage XP-12, in which all of the cytosine residues are replaced by 5-methylcytosine. Ehrlich reports that methylation of cytosine increases the melting temperature of XP-12 over non-methylated XP-12. Hua calculates the increase in melting temperature of methylated Z-DNA over unmethylated B-DNA using a modified self-consistent effective photon approximation. Tyagi '097 describes a probe capable of undergoing a conformational change upon interacting with a target in an assay

preferably having a fluorophore and a quencher. Finally, Coull describes protein nucleic acid (PNA) molecular beacons in place of deoxyribonucleic acid (DNA) molecular beacons comprising self-complementary arm segments and flexible linkages that promote intramolecular or intermolecular interactions.

As stated in the Amendment and Reply dated October 23, 2002, which is hereby incorporated by reference, the Examiner has failed to make out a *prima facie* case of obviousness. There is no teaching or suggestion in the prior art, including the cited references, to combine the references to arrive at the claimed invention. (See, e.g., *In re Geiger*, 815 F.2d 686, 688, 2 U.S.P.Q.2d 1276, 1278 (Fed. Cir. 1987), *In re Gorman*, 18 U.S.P.Q.2d 1885, 1888 (Fed. Cir. 1991), and *In re Dembiczak* (50 USPQ2d 1614 (Fed. Cir. 1999)).

As his basis for motivation to combine the references, the Examiner states that "assays which are able to determine a predisposition to cancer, onset of cancer and such are of real concern to the scientific community" (Office Action, page 9, last paragraph). Although Applicant agrees that finding ways to predict or diagnose cancer are of "real concern to the scientific community," such concern is not a legitimate basis for an obviousness rejection. It is well established that obvious to try is not the standard under Section 103 (see, e.g., *In re O'Farrell*, 853 F.2d 894, 903 (Fed. Cir. 1988)). In the instant case, the Examiner has failed to cite to any specific passages in the cited references which provide motivation to combine those references, nor to any other sources for such motivation to produce Applicant's invention. Absent such motivation, the Examiner has failed to establish a prima facie case of obviousness. In other words, although it may have been obvious to vary all parameters of the cited references or try each of numerous possible choices and combinations of methodologies described therein until one possibly arrived at a successful result, such "obvious to try" experimentation is not the proper standard of obviousness under 35 U.S.C. § 103.

Moreover, the combination of all five references fails to teach or suggest the claimed invention. None of the references teach the element of detecting methylated nucleic acids, or the element of an oligonucleotide sequence containing a region that is susceptible to methylation. Only in Applicant's disclosure is the element of detecting methylated nucleic acids found.

In light of the foregoing arguments, the Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

B) Rejection of Claims 7, 10, 12 and 13 under 35 U.S.C. § 103(a)

Claims 7, 10, 12 and 13 are rejected under 35 U.S.C. § 103(a) over Elsas, in view of either Ehrlich or Hua and in further view of either Tyagi '097 as applied to Claims 1-6, 14-19 above, and further in view of Herman, et al., U.S. Patent 6,265,171 (hereinafter "Herman").

The Examiner states that it would have been obvious to use the method of Elsas, Ehrlich or Hua and Tyagi `097 or Coull in view of the teaching of Herman in which genes including GSTpi and calcitonin are differentially methylated at CpG islands in neoplastic versus normal tissue. Applicant respectfully disagrees with the grounds of the rejection.

As discussed above, there is no teaching or suggestion in Elsas, Ehrlich, Hua, Tyagi '097 and Coull to detect the presence of methylated nucleic acids by the methods of Applicant's claims. Nor does Herman supply the deficiencies. Herman is silent with respect to detecting methylated nucleic acids using oligonucleotide probes, as well as providing motivation to combine the specific references to achieve Applicant's claimed invention.

Claims 7, 10, 12 and 13 depend from independent Claim 1. Because Claim 1 is unobvious over the cited references, as discussed above, dependent claims 7, 10, 12 and 13 are similarly unobvious. Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

C) Rejection of Claims 7 and 8 under 35 U.S.C. § 103(a)

Claims 7 and 8 are rejected under 35 U.S.C. § 103(a) over Elsas in view of either Ehrlich or Hua and in further view of either Tyagi '097 or Coull as applied to Claims 1-6, 14-19 above, and further in view of Kay, et al., *Leukemia and Lymphoma*, Vol. 24, pages 211-220, 1977 (hereinafter "Kay").

The Examiner states that it would have been obvious to use the method of Elsas, Ehrich or Hua and Tyagi '097 or Coull, in view of the teaching of Kay, in which the Myf-3 gene is abnormally hypermethylated. Applicant respectfully disagrees with the grounds of the rejection.

Kay teaches that the Myf-3 gene can be hypermethylated and that the hypermethylated status of the Myf-3 gene may provide new diagnostic indicators of malignancy. Kay is silent with respect to detecting methylated nucleic acids using oligonucleotide probes, as well as regarding motivation to combine the particular references cited by the Examiner to achieve Applicant's claimed invention. The only motivation that Kay provides is the desire for more extensive studies in the diagnosis of malignant lymphomas. Again, as discussed above, "obvious to try" is not the proper standard under 35 U.S.C. § 103.

Claims 7 and 8 depend from independent Claim 1. Because the combination of references fails to render independent claim 1 obvious, dependent claims 7 and 8 are similarly unobvious. Applicant respectfully requests that the rejection under 35 U.S.C. § 103(a) be reconsidered and withdrawn.

D) Rejection of Claim 18 under 35 U.S.C. § 103(a)

Claim 18 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Tyagi et al. (*Nature Biotech.* (1996), **14**:303-308) in view of Ahern (*The Scientist* (1995), **9**(15):20).

The Examiner states that it would have been obvious to have modified the teachings of Tyagi with the teachings of Ahern to incorporate the necessary reagents into a packaged kit. More specifically, "[t]he ordinary artisan would have been motivated to have packaged the primers, probes, and reagents of Tyagi into a kit, as taught by Ahern for the express purpose of saving time and money."

As discussed in Section I above, Claim 18 requires a loop sequence which has a "region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation." Although the Tyagi reference teaches a

molecular beacon probe which detects complementary targets, wherein the molecular beacon comprises a fluorophore moiety and a quencher, it does not teach an oligonucleotide probe having "a loop sequence comprising a nucleotide region complimentary to a region of the nucleic acid sample, wherein the region of the nucleic acid sample is susceptible to methylation," as required by Claim 18. Nor does Ahern, which teaches reagent kits for scientists, supply the deficiencies of Tyagi et al. Thus, because the references do not teach every element of Claim 18, they do not obviate the claimed invention.

III. Conclusion

Applicant submits that in view of the foregoing amendment and remarks, all issues relevant to patentability raised in the Office Action have been addressed. Applicant respectfully requests the withdrawal of rejections over the claims of the present invention.

Respectfully submitted,

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MARKED-UP VERSION OF AMENDMENTS:

Claim Amendments Under 37 C.F.R. § 1.121(c)(1)(ii)

Please amend the claims as follows:

18. (Amended) A kit for distinguishing methylated and non-methylated nucleic acid sequences, comprising a labeled oligonucleotide probe [; said labeled oligonucleotide probe characterized in that:

(a) it comprises a first stem labeled with a fluorophore moiety, a loop sequence having a region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation, and a second stem labeled with a quencher moiety that is capable of quenching the fluorophore moiety when in spatial proximity to the fluorophore moiety; and

(b) the nucleotides forming the first stem are capable of moving into spatial proximity with the nucleotides forming the second stem when the oligonucleotide sequence is dissociated from the nucleic acid sample.]

wherein said labeled oligonucleotide probe comprises a fluorophore moiety, a loop sequence, and a quencher moiety, and wherein said loop sequence has a region of nucleotides complementary to at least a region of the nucleic acid sample, which region of the nucleic acid sample is susceptible to methylation.